



Objectives

- Understand the most common tests used by the clinical bacteriology laboratory for identification and susceptibility testing of clinical isolates.
- Understand the classes of antimicrobial agents and their potential uses.
- Describe mechanisms for development of antibiotic resistance in bacteria, including carbapenem resistance.
- Describe the laboratory tests used to detect carbapenem resistance and the challenges involved in the interpretation of the laboratory data.
- Describe the role of biosafety in protecting the healthcare provider.
- Explain the relationship between hazard, risk, and risk assessment.
- Understand the Antibiotic Resistance Laboratory Network initiative.



The Basics of Clinical Bacteriology

➤ Bacteria are classified by various characteristics which allow them to be identified by the laboratory:

1. Gram stain appearance and shape:

GRAM-POSITIVE

GRAM-NEGATIVE

Fixation

Crystal Violet

Iodine Treatment

Decolorisation

Counter stain with Safranin

Bacilli

Cocci

Spirilli

Gram Positive Cocci in chains (purple)

Gram Positive Diplococci (purple)

Gram Negative Diplococci (red/pink)

Gram Negative Rods (red/pink)

Gram Positive Cocci in Clusters (purple)

Gram Positive Rods (purple)

The logo for the Louisiana Department of Health, featuring a stylized cross and the text "LOUISIANA DEPARTMENT OF HEALTH".

The Basics of Clinical Bacteriology

2. Types of culture media exhibiting growth:

3. Colony shape and size:

Nutrient Agar

MacConkey Agar

MacConkey Agar

XLD Agar

Staphylococcus aureus

Escherichia coli

Pseudomonas aeruginosa

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The Basics of Clinical Bacteriology

4. Atmospheric requirements for bacterial growth:



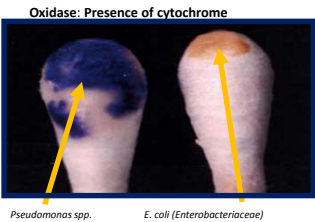
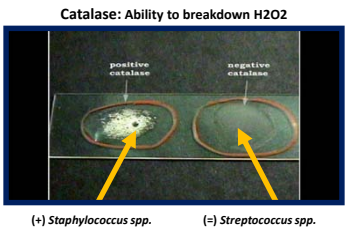
- **CO₂** – *Neisseria spp.*, *Haemophilus spp.*, *Streptococcus pneumonia*
- **Microaerophilic (reduced O₂)** – *Campylobacter spp.*
- **Anaerobic (lack of O₂)** – *Clostridium difficile*



The Basics of Clinical Bacteriology

5. Organism Identification:

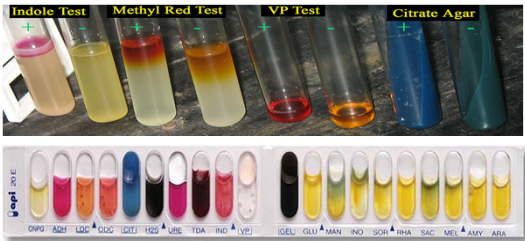
➤ **Spot tests** – rapid biochemical tests which can be used to rule in/out various groups of organisms



The Basics of Clinical Bacteriology

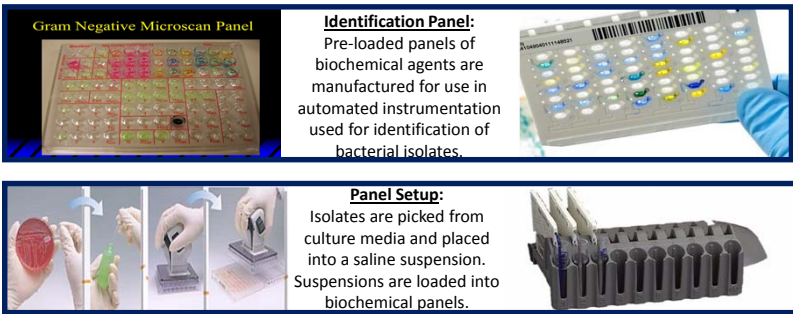
5. Organism Identification:

➤ **Biochemical characterization (manual)** – isolate suspensions are incubated with a selection of various biochemical agents. Collective results of biochemical tests are compared to a database of organisms with known biochemical reactions in order to determine the identification of the isolate.



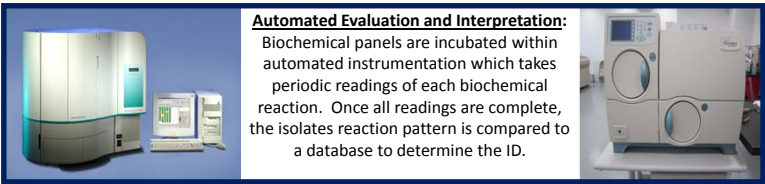
The Basics of Clinical Bacteriology

➤ **Biochemical characterization (automated)** – manual reads replaced by automated reads and isolates are identified using an onboard database.



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The Basics of Clinical Bacteriology

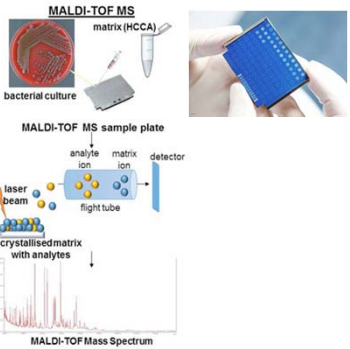
➤ **MALDI-TOF** – Matrix Assisted Laser Desorption/Ionization Time of Flight



Target Plate Preparation:
Bacterial colony suspended in formic acid on a stainless steel target plate and overlaid with organic matrix compound.

Desorption/Ionization:
Matrix/suspended colony crystallize and are ionized to generate charged bacterial protein fragments.

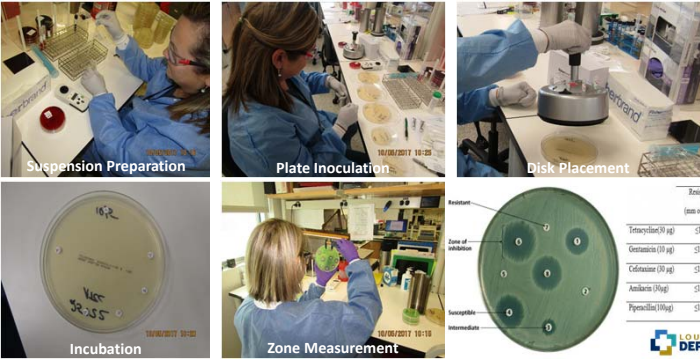
Time of Flight:
Ionized, charged proteins travel through a vacuum tube and hit detector to produce unique spectra which are compared to a database of spectra.



The Basics of Clinical Bacteriology

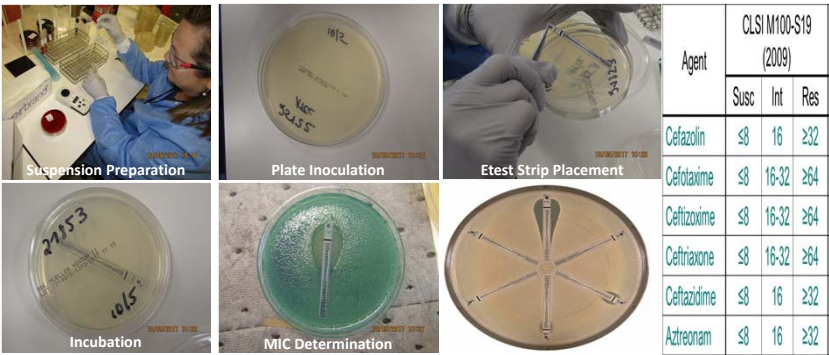
6. **Antimicrobial Susceptibility Testing (AST):** *Determining the inhibitory effects of antimicrobial concentration on organism growth*

➤ **Disk Diffusion (manual)** - application of paper discs containing antimicrobial agents



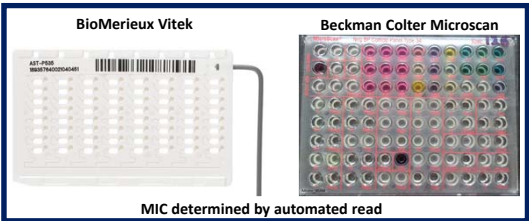
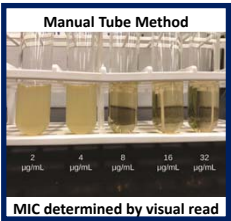
The Basics of Clinical Bacteriology

➤ **Etest (manual)** - Application of a plastic strip coated with a gradient of an antimicrobial agent



The Basics of Clinical Bacteriology

➤ **Broth Microdilution (manual or automated)** - Incubation of an isolate suspension in a known concentration of an antimicrobial agent



Minimum Inhibitory Concentration (MIC):
The lowest concentration of an antibiotic which will inhibit the in vitro growth of an organism

Breakpoint:

A breakpoint is a chosen concentration (µg/mL) of an antibiotic which defines whether a species of bacteria is susceptible, intermediate or resistant to antimicrobial therapy. Establishment of breakpoints takes into account normal MIC values of wild type organisms, outcomes from clinical infections, and achievable in vivo antimicrobial concentrations.

In order for an antimicrobial therapy to be effective, the MIC must fall within the Susceptible (S) or Intermediate (I) range as established by the breakpoints.



Antibiotics

1. Antimicrobial drugs are used in the treatment and prevention of bacterial infections

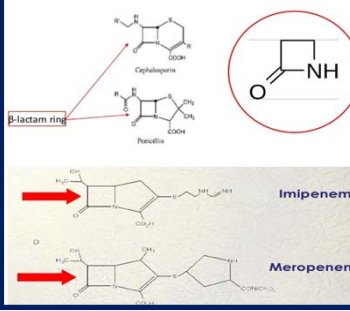
CLASSIFICATION		ANTIBIOTICS		
Penicillins	Natural Penicillins (narrow spectrum)	Penicillase – Sensible		
	Aminopenicillins (broad spectrum)	Penicillin G: Na, K, Procaine, Benzathine (IV, IM)		
	Antipseudomonal (extended spectrum)	Penicillin V, NO		
		Ampicillin		
Cephalosporins	1 st Generation	Penicillase – Resistant (very narrow spectrum)		
	2 nd Generation	Nafcillin		
	3 rd Generation	Oxacillin		
	4 th Generation	Dicloxacillin		
Carbapenems	1 st Generation	Carbapenemase – Sensible		
	2 nd Generation	Meropenem		
	3 rd Generation	Ertapenem		
	4 th Generation	Imipenem + Cilastatin		
Aminoglycosides	1 st Generation	Tetracycline		
	2 nd Generation	Doxycycline		
	3 rd Generation	Minocycline		
	4 th Generation	Clarithromycin		
Macrolides	1 st Generation	Clarithromycin		
	2 nd Generation	Clarithromycin		
	3 rd Generation	Clarithromycin		
	4 th Generation	Clarithromycin		
Fluoroquinolones	1 st Generation	Ciprofloxacin		
	2 nd Generation	Moxifloxacin		
	3 rd Generation	Moxifloxacin		
	4 th Generation	Moxifloxacin		
Sulfonamides	1 st Generation	Sulfamethoxazole		
	2 nd Generation	Trimethoprim (TMP)		
	3 rd Generation	Trimethoprim (TMP)		
	4 th Generation	Trimethoprim (TMP)		
DHFR Inhibitors	1 st Generation	Trimethoprim (TMP)		
	2 nd Generation	Trimethoprim (TMP)		
	3 rd Generation	Trimethoprim (TMP)		
	4 th Generation	Trimethoprim (TMP)		
Nitroimidazole	1 st Generation	Metronidazole		
	2 nd Generation	Metronidazole		
	3 rd Generation	Metronidazole		
	4 th Generation	Metronidazole		



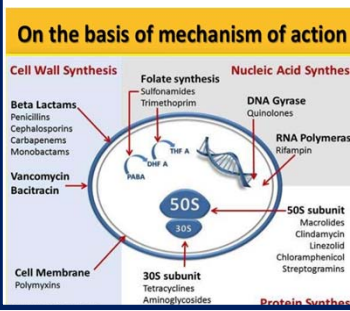
Antibiotics

2. Classification: Based on structure and activity

Structure – Antibiotics are grouped into classes based on their chemical structure.



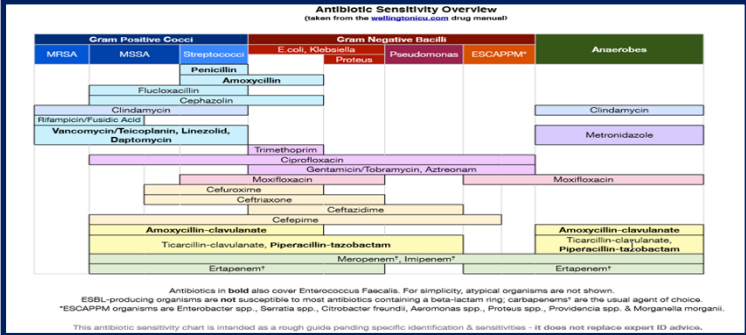
Activity – Antimicrobials inhibit bacterial growth by a variety of mechanisms



Antibiotics

➤ **Spectrum**

Narrow - Used for treatment of a specifically targeted group of bacteria (Example: Vancomycin)
Broad - Used for treatment of a wide variety of bacteria (Example: Ceftriaxone)

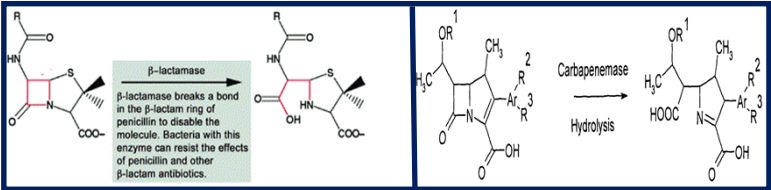


Antibiotic Resistance

1. Resistance mechanisms:

➤ The **Production of enzymes** which breakdown antibiotics. Name of the enzyme (which usually ends in *-ase*) is dependent on its target.

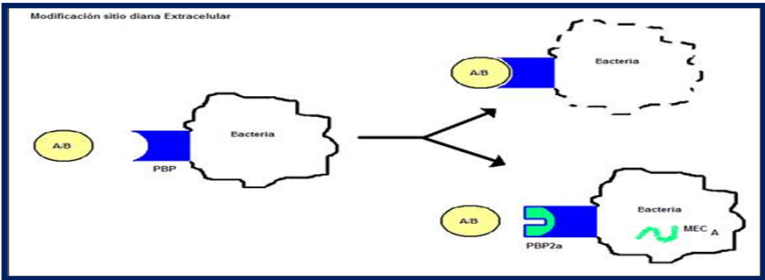
- **Beta-lactamases** – breakdown Beta-lactams (i.e. Penicillin, Ampicillin)
- **Cephalosporinases** – breakdown Cephalosporins (i.e. Cefazolin)
- **Carbapenemases** – breakdown Carbapenems, Cephalosporins, and Beta-lactams



Antibiotic Resistance

➤ **Changes in an organisms cell structure** create barriers to antimicrobial action.

- **Changes in the binding site of the antibiotic** – Penicillin binding protein 2a (PBP2a) prevents the binding of antibiotics such as penicillin and methicillin in resistant species such as Methicillin Resistant Staphylococcus aureus (MRSA).



Antibiotic Resistance

2. Resistance Types:

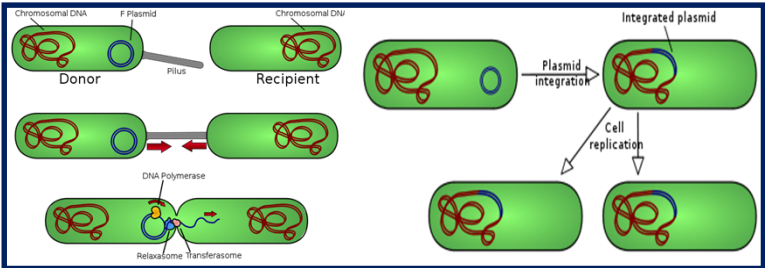
➤ **Intrinsic Resistance** - Natural resistance in bacteria due to cell structure or natural enzyme production. Resistance due to natural enzyme production may be enhanced due to overexpression of resistance genes during treatment (Example: AmpC in *Pseudomonas* and *Acinetobacter* spp.).

Organisms	Antibiotics	Mechanism
Gram-positive bacteria	Aztreonam (beta-lactam)	Lack of penicillin binding proteins which can effectively bind aztreonam
Gram-negative bacteria	Vancomycin	Large molecule of vancomycin is unable to penetrate outer membrane of G-ve bacteria
Klebsiella spp.	Ampicillin	β -lactamases produced by the bacteria destroy ampicillin before it reaches the PBP targets
Stenotrophomonas maltophilia	Imipenem	β -lactamase produced by the bacteria destroy imipenem before it bind with PBP target.
Lactobacillus and Leuconostoc	Vancomycin	Unable to bind with cell wall precursor
Pseudomonas aeruginosa	Sulfonamides, trimethoprim, tetracycline, chloramphenicol	In-effective intracellular concentrations of antibiotics due to lack of uptake
Enterococcus spp.	Aminoglycosides	Limited uptake of aminoglycosides by protein of electron transport chain
	β -lactam antibiotics like penicillin, cephalosporins and monobactam	Lack of penicillin binding proteins

Antibiotic Resistance

2. Resistance Types:

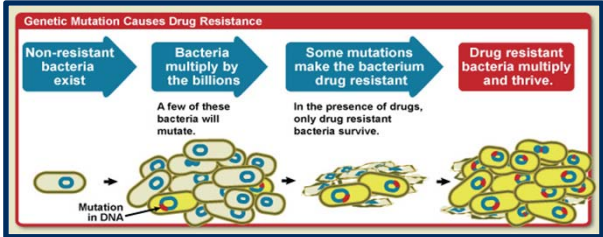
➤ **Acquired Resistance** - Resistance genes are transported from one bacteria to another via mobile elements called plasmids. This allows bacteria that do not naturally possess genes that confer resistance to accept those genes from bacteria that do possess them (Example: ESBL's in *E. coli* and *Klebsiella* spp.).



Antibiotic Resistance

3. Selective Pressure:

- The influence of antimicrobial agents on natural selection to promote one group of organisms over another
- Kills susceptible bacteria, allowing for the survival and multiplication of antimicrobial resistant bacteria



Antibiotic Resistance

4. Levels of Resistance:

- Resistance is defined by one key drug (i.e. MRSA and VRE)
- Resistance to multiple classes of antibiotics (i.e. resistance to Penicilins, Cephalosporins, Carbapenems, Fluoroquinolones, and Aminoglycosides seen in members of *Enterobacteriaceae*, and in *Pseudomonas* and *Acinetobacter* spp.

Bacteria	Acronyms	Antibiotic Resistance
Staphylococcus aureus	MRSA	Methicillin Resistance
Enterococcus faecalis/faecium	VRE	Vancomycin Resistance
Enterobacteriaceae	ESBL	Extended Spectrum Beta-lactam Resistance
Enterobacteriaceae	CRE	Carbapenem Resistance
Pseudomonas/Acinetobacter spp.	MDR	Multi-Drug Resistance

Antibiotic Resistance

5. Extended Spectrum Beta-Lactamases (ESBL's):

- More than 200 originating from more than 30 different countries
- Plasmid mediated and confer bacterial resistance to the penicillins, first, second, and third-generation cephalosporins, and aztreonam

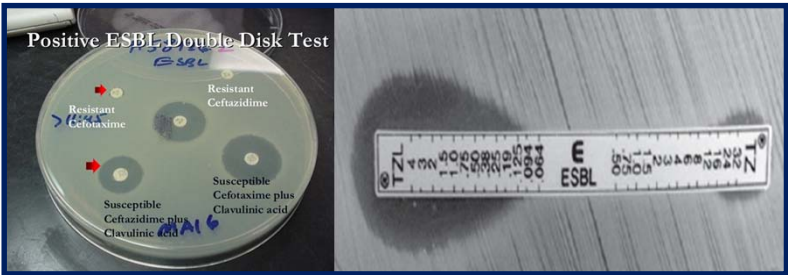
Common Group History:

- **TEM-1**, first plasmid mediated ESBL discovered in Greece in the 1960's and SHV-1 a variant of TEM-1: resistance to penicillin's and early generation cephalosporins
- **SHV-2**, discovered in Germany in early 1980's after introduction of third-generation cephalosporins: resistance to extended spectrum (3rd generation) cephalosporins (ESBL)
- **CTX-M**, named for potent activity against cefotaxime and accounts for second largest group of ESBL's mostly found in *E. coli*
- **OXA**, originally discovered in *Pseudomonas* spp with spread to *Enterobacteriaceae* via plasmids: Resistance common to *Pseudomonas* spp. (i.e. cefotaxime, ceftazidime, and aztreonam)

Antibiotic Resistance

- **ESBL Detection** - (Note: only valid for Klebsiella, E. coli, and Proteus)

- **Manual methods:** Susceptibility testing for 3rd generation Cephalosporins with and without the presence of Clavulanic Acid yields difference of ≥ 5 mm in zone size (disk diffusion) or MIC's display a ratio \geq to a known value (Etest).



Antibiotic Resistance

- **ESBL Detection** - (Note: only valid for Klebsiella, E. coli, and Proteus)
- **Automated Methods:** Commercially produced antimicrobial panels used in automated instruments assess the antibacterial activity of Cefepime, Cefotaxime, and Ceftazidime with and without the presence of Clavulanic Acid. Results are interpreted by onboard software.



Antibiotic Resistance

- **Interpretation of ESBL screening results -**
- **Clinical and Laboratory Standards Institute (CLSI) interpretive standards prior to January 2010:** If screening results are positive report all penicillins, cephalosporins, and aztreonam as resistant (R) regardless of values obtained.
 - **Clinical and Laboratory Standards Institute (CLSI) interpretive standards after January 2010 (not FDA approved):** Breakpoints lowered for cephalosporins and aztreonam eliminating need for ESBL screen. Results reported as tested.

E. coli with Positive ESBL Screen

Agent	Results	CLSI M100-S19 (2009)				CLSI M100-S20 (2010)			
		S	I	R	Interp	S	I	R	Interp
Cefazolin	32	≤8	16	≥32	R	≤1	2	≥4	R
Cefotaxime	8	≤8	16-32	≥64	R	≤1	2	≥4	R
Ceftriaxone	2	≤8	16-32	≥64	R	≤1	2	≥4	I
Ceftazidime	4	≤8	16	≥32	R	≤4	8	≥16	S
Aztreonam	16	≤8	16	≥32	R	≤4	8	≥16	R

Antibiotic Resistance

- **ESBL Treatment -**
- β-lactam/ β-lactamase inhibitor combinations (ex. Piperacillin-tazobactam) are not considered optimal therapy for serious infections due to ESBL producers due to a history of poor clinical outcomes
 - Therapeutic options are limited to carbapenems, colistin, polymyxin, temocillin, tigecycline for **serious infections**.
 - However uncomplicated infections like urinary tract infections can be managed with a variety of antibiotics including oral antibiotics like trimethoprim, nitrofurantoin, or intravenous agents like aminoglycoside (gentamicin, amikacin).
 - Carbapenems are the drugs of choice for serious infections with ESBL producers.
 - There is also concern that misuse of carbapenems in uncomplicated cases will result in development of carbapenem resistance.

Antibiotic Resistance

6. **Carbapenem Resistance:**
- Carbapenems are extremely broad spectrum antimicrobial agents that should be reserved for severe, complicated infections. Carbapenem resistance significantly limits treatment options for life-threatening infections.
 - Resistance to Carbapenems in some species is intrinsic (ex. Stenotrophomonas maltophilia – metallo-beta-lactamase).
 - Gram positive organisms typically develop Carbapenem resistance through mutations-derived changes to their penicillin binding proteins (PBP's).
 - Some gram negative organisms develop Carbapenem resistance via genetic and structural changes -
 - Other gram negative organisms develop Carbapenem resistance by acquiring an enzyme called a carbapenemase via plasmid transfer.

Antibiotic Resistance

7. Carbapenemases:

- Hydrolyze **all or almost all** beta-lactams
- Plasmids carrying carbapenemase genes commonly carry additional resistance genes (e.g. ESBL).
- The most effective carbapenemases, in terms of carbapenem hydrolysis and geographical spread, are KPC, VIM, IMP, NDM and OXA-48

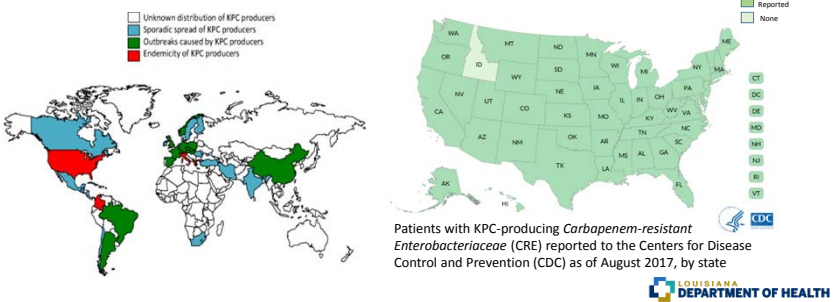
Ambler Classification of β -lactamases

Ambler Class	A	B	C	D
Active Site	Serine	Metallo (inc. binding fluid)	Serine	Serine
Enzyme Type	TEM, SHV, CTX-M, KPC	NMD-1, IMP, VIM	AmpC, CMY	OXA
Host Organisms	Enterobacteriaceae and Non-fermenters	Enterobacteriaceae and Non-fermenters	Enterobacter spp. Citrobacter spp.	Enterobacteriaceae and Non-fermenters
Substrates	Ampicillins; cephalosporins; penicillins; 3 rd generation cephalosporins; carbapenems	All β -lactams	Cepharmycins; 3 rd generation cephalosporins	Cloxacillin; Extended-spectrum cephalosporins; carbapenems

Antibiotic Resistance

➤KPC – Class A: MOST COMMON

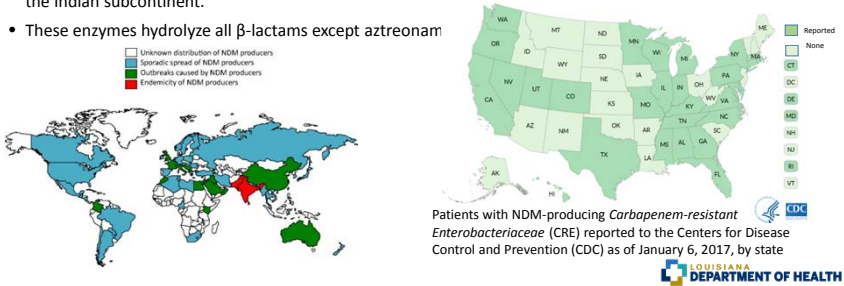
- First found in the late 1990s in the New York City
- First found in *Klebsiella pneumoniae* hence the name KPC
- Spread to bordering states, Latin America, Israel, Greece, and southern Europe



Antibiotic Resistance

➤NDM-1 – Class B:

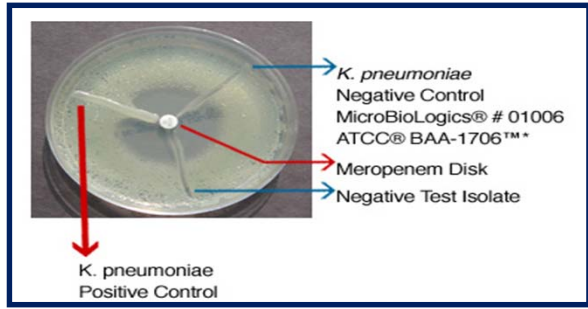
- New Delhi metallo-beta-lactamase detected in 2008 in India
- NDM-1 producers have been identified mainly in the United Kingdom, India, and Pakistan but have also been isolated in many countries in Europe, Asia, Africa, Australia, and North America
- Most of the patients infected by NDM-1 producers are from India, Pakistan, or Bangladesh or have traveled in the Indian subcontinent.
- These enzymes hydrolyze all β -lactams except aztreonam



Antibiotic Resistance

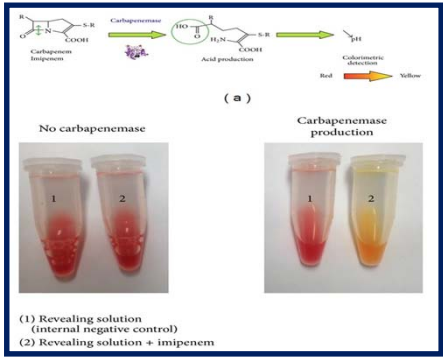
8. Carbapenemase Detection: Isolates exhibiting carbapenem resistance using manual (disk diffusion/Etest/broth microdilution) or automated methods may be confirmed by the following methods

- Modified Hodge Test – Evaluate the growth of a carbapenem susceptible E. coli isolate in the presence of a possible carbapenemase producing organism



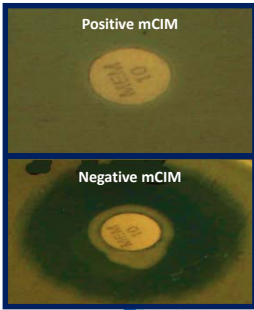
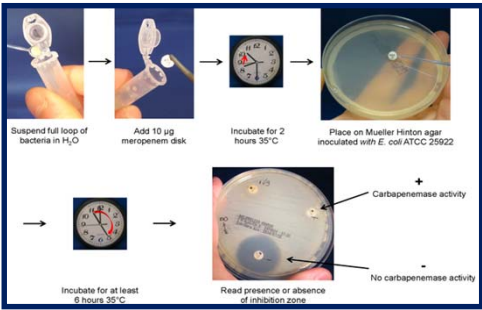
Antibiotic Resistance

➤ **Carba NP test** – Uses principle of imipenem hydrolysis and a colorimetric pH indicator to detect the presence of carbapenemases



Antibiotic Resistance

➤ **Modified Carbapenem Inactivation Method (mCIM)**- Evaluate the growth of a carbapenem susceptible E. coli isolate in the presence of a carbapenem disk that has been exposed a possible carbapenemase producer

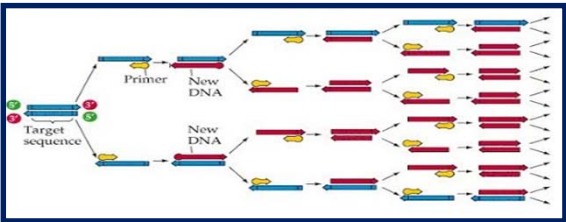


Antibiotic Resistance

9. **Molecular Characterization:** Use of PCR analysis for the detection and differentiation of the KPC, NDM, VIM, OXA-48, and IMP gene sequences associated with carbapenem non-susceptible bacterial isolates.

➤ **Polymerase Chain Reaction (PCR)** -

- Technique that permits the analysis of any short sequence of DNA (or RNA) even in samples containing only minute quantities of DNA or RNA.
- PCR is used to reproduce (amplify) selected sections of DNA or RNA for analysis.



Antibiotic Resistance

➤ **Cepheid GenXpert Carba R Assay** -

- PCR analysis performed on an automated platform.
- Detects gene sequences for KPC, NDM, VIM, OXA-48, and IMP



Antibiotic Resistance

10. **Interpretation of Carbapenem Susceptibility Results:**
- **Clinical and Laboratory Standards Institute (CLSI) interpretive standards prior to June 2010:**
If carbapenemase production is suspected in isolates of Enterobacteriaceae, laboratories should perform a Modified Hodge Test, Carba NP Test, mCIM test, or a molecular assay to confirm the presence of a carbapenemase.
 - **Clinical and Laboratory Standards Institute (CLSI) interpretive standards after June 2010 (not FDA approved):** Breakpoints lowered for carbapenems **eliminating need for confirmation** by Modified Hodge Test, Carba NP Test, mCIM test, or a molecular assay. Results reported as tested.

Agent	CLSI M100-S19 (2009)			CLSI M100-S20 (2010) Supplement		
	Susc	Int	Res	Susc	Int	Res
Doripenem	-	-	-	≤1	2	≥4
Ertapenem	≤2	4	≥8	≤0.25	0.5	≥1
Imipenem	≤4	8	≥16	≤1	2	≥4
Meropenem	≤4	8	≥16	≤1	2	≥4



Resistance Profile: ESBL Producer vs. CRE

Organism Identification: *E. coli*

ESBL Producer		CRE	
Antimicrobial	Interpretation	Antimicrobial	Interpretation
Cefazolin	R	Cefazolin	R
Ceftriaxone	R	Ceftriaxone	R
Ceftazidime	R	Ceftazidime	R
Cefepime	R	Cefepime	R
Aztreonam	R	Aztreonam	R
Piperacillin-Tazobactam	S	Piperacillin-Tazobactam	R
Meropenem	S	Meropenem	R
Gentamycin	S	Gentamycin	S



Antibiotic Resistance

11. **CRE Treatment:** Clinicians have been forced to re-evaluate the use of agents which have been historically rarely used due to efficacy and/or toxicity concerns, such as polymyxins and aminoglycosides. Additional CRE treatment strategies include optimization of dosing regimens and combination therapy.

- **Carbapenems**
 - High mortality rates for patients treated with high dose carbapenem monotherapy
 - High dose carbapenem combination therapy with a second agent such as Polymyxin B or an Aminoglycoside shown to be effective even with isolates with an MIC > 8 µg/mL
- **Polymyxins**
 - Polymyxin B has advantages over Colistin (Polymyxin E) due to ability to achieve higher serum concentrations in a short period of time
 - Polymyxin monotherapy is associated with rapid resistance development but is effective if used in combination with high dose carbapenems or aminoglycosides
 - Prolonged therapy is associated with nephrotoxicity and neurotoxicity
- **Tigecycline**
 - Tigecycline monotherapy is associated with high mortality rates
 - Tigecycline combination therapy is effective with high dose carbapenem, aminoglycosides, or colistin



Antibiotic Resistance

11. **CRE Treatment:**

- **Aminoglycosides -**
 - Gentamycin most active against carbapenem resistant *K. pneumoniae*
 - Amikacin is most active against other CRE
 - Several clinical studies have shown that aminoglycoside combination therapy is most effective if used along with high dose carbapenems
- **Emerging Treatment -**
 - Ceftazidime-avibactam – 3rd generation Cephalosporin and novel beta-lactamase inhibitor with activity against class A, B, and D beta-lactamases but no activity against class B MBL's (ex. NDM-1)
 - Ceftaroline-avibactam - 3rd generation Cephalosporin and novel beta-lactamase inhibitor with activity against class A, B, and D beta-lactamases but no activity against class B MBL's (ex. NDM-1)

Note: Combination therapies used to treat CRE infections are associated with an increased risk for the development of *Clostridium difficile* infection and/or colonization and adverse effects such as nephrotoxicity.



What is Biosafety?

The discipline addressing the safe handling and containment of infectious microorganisms and hazardous biological materials.

- Risk Assessment
- Containment



Risk Assessment (RA) - is a *process* that involves *hazard identification and hazard control*

➤ What is a *hazard*?

A *hazard* is something that is intrinsically dangerous such as an object, a chemical, an infectious agent, or a situation.

Risk Assessment

What is *RISK*?

- “Possibility of loss, injury, disease, or death.” Webster’s Medical Desk Dictionary (1986)
- “The probability that exposure to a hazard will lead to a negative consequence.” David Ropeik, George Gray (2002)
 - A *prediction*
- “To risk living is to risk dying.” Anonymous

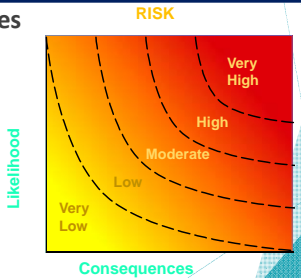
Risk

The likelihood that an adverse event involving a specific hazard will occur and the consequences of the occurrence.

➤ Risk = Likelihood x Consequences

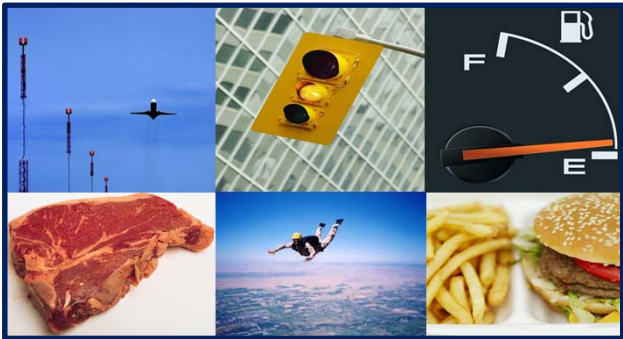
➤ Situational

- Risk rating is dependent on what, who, where



Risk Assessment Is Not New

➤ *We conduct risk assessments all the time...*



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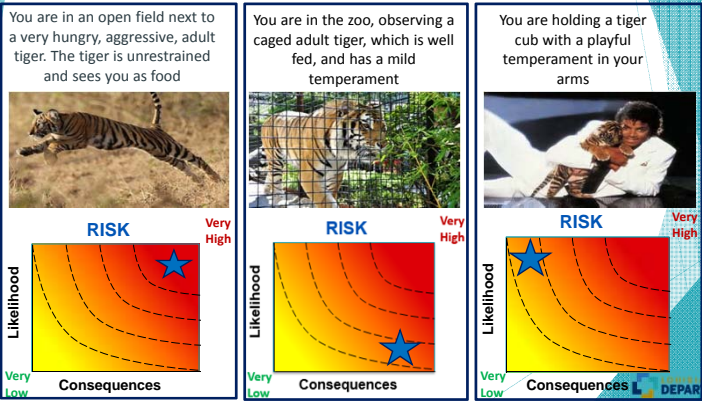
What is the **risk** of being attacked by a tiger?

What do you need to know to answer this question?

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For the For the following scenarios

Draw a STAR where the **risk** would fall on each graph:



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Q: *Why Do Risk Assessments?*

➤ *As a healthcare worker you work with or handle infectious materials every day.*



A: Because of where you work and what you do...

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Why Do Risk Assessments?

To **reduce** and **minimize** the risk of exposure to workers and the environment

But remember:

Risk is never zero!

Why Do Risk Assessments?

To **prevent** healthcare provider-acquired infections due to:

- Direct contact (spills/splashes) to mucous membranes
- Inhalation of aerosols
- Percutaneous inoculation from cuts, sharps, vectors, non-intact skin
- Ingestion
- Indirect contact (contamination from fomites)

Risk Assessment (RA)

Risk assessment is simply a process determining:

➤ **What are we doing?**

- **identify** the hazards (agent, procedures, and staff)

➤ **What can go wrong?**

- **evaluate** the risks and consequences of exposure

➤ **What do we have in place or need in place to prevent our staff from exposure?**

- **determine** controls (mitigate)
- **implement** controls
- **review and adjust** = CONTINUOUS IMPROVEMENT PROCESS

Risk assessment is the **basis** of a biosafety program.



Steps of RA

1. Gather information and identify the potential hazard
2. Evaluate and prioritize the risk (likelihood and consequence)
3. Determine what additional safety precautions (controls) are needed to reduce the risk (mitigation)
4. Implement controls
5. Review and evaluate effectiveness, adjust

Pathogen Safety Data Sheet (PSDS)

Public Health Agency of Canada

Agency Information

NEISSERIA MENINGITIDIS

PATHOGEN SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

SYNOPSIS OR CROSS REFERENCE

CHARACTERISTICS

SECTION II - HAZARD IDENTIFICATION

PATHOGENICITY/TOXICITY

Information

Reports & Publications

Transparency

Pathogen Safety Data Sheets and Risk Assessment

Important Note: Pathogen Safety Data Sheets (PSDSs) are technical documents used by individuals working with pathogens in the laboratory. To obtain any other information about infectious diseases, please visit [Infectious Diseases](#)

Laboratory Biosafety and Biosecurity

Pathogen Safety Data Sheets (PSDSs) (previously titled Material Safety Data Sheets for infectious substances) are technical documents that describe the hazardous properties of a human pathogen and provide recommendations for work involving these agents in a laboratory setting. These documents have been produced by the Public Health Agency of Canada (PHAC) as educational and information resources for laboratory personnel working with infectious substances.

Please note that work involving pathogens in Canada may require compliance with international, national, and provincial laws and guidelines.

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php>

Conducting a Risk Assessment

Who Does Risk Assessments?

Ideally, a multidisciplinary team:

- Healthcare Provider (HCP)
- Managers/supervisors
- Health and safety specialists (biosafety, occupational health)
- Facilities staff
- Infection Control Preventionists

When?

Ideally, at regular intervals:

- More frequently in problem areas
- When there is an incident, accident, or exposure
- When changes occur:
 - Move, renovation, or new facility
 - New infectious agent or reagent
 - New piece of equipment, technique, or procedure
 - New scientific information available

Biosafety Risk Assessment Example

Specimen Collection and Transport: (continued)					
Preanalytic: Procedure	Process Step	Potential Hazards	Initial Risk Level	Control (Mitigation)	Residual Risk Level
<input checked="" type="checkbox"/> Perform Phlebotomy	<ol style="list-style-type: none">1) Confirm patient identification (2 identifiers)2) Disinfect phlebotomy site and any blood culture media.3) Collect blood specimen into various collection tubes or bottles for laboratory testing via venipuncture (Follow correct order of draw).4) When draw is completed cap the needle using the needle safety capping device. Never manually recap a needle holding the cap in your hand.5) If a syringe draw, transfer blood from the syringe into collection tubes or bottles for laboratory testing using a safety transfer device6) Dispose of contaminated needle puncture device into a sharps container.7) Apply pressure to patient's venipuncture site until bleeding stops.8) Cover venipuncture site with gauze and tape or bandage.9) Label specimens.	<ul style="list-style-type: none">• Needle stick with a clean or contaminated needle during specimen collection, or when employing safety capping device• Spill, splash or spray of blood into mouth, mucous membrane or onto non-intact skin	Moderate	<p>Required:</p> <ul style="list-style-type: none"><input type="checkbox"/> Follow written phlebotomy policy.<input type="checkbox"/> Wear PPE: lab coat and gloves. Wear additional PPE: N-95 respirator/PAPR and/or disposable gown if patient is known or suspected of having an infectious disease transmitted by aerosols or droplets (i.e. TB, MERS, SARS, Avian Influenza, mumps, measles, etc.).<input type="checkbox"/> Use needles with safety capping devices.<input type="checkbox"/> Dispose of used needles in a rigid sharps container. <p>Optimal:</p> <ul style="list-style-type: none"><input type="checkbox"/> Wear eye protection.<input type="checkbox"/> Ask patient about travel outside the USA in the past 30 days.<input type="checkbox"/> If patient has a known contagious disease/condition such as MRSA, VRE, MRO, CRE, C. diff, mumps, measles, etc., disinfect the drawing area before using the area for another patient.	Low
Comments:					

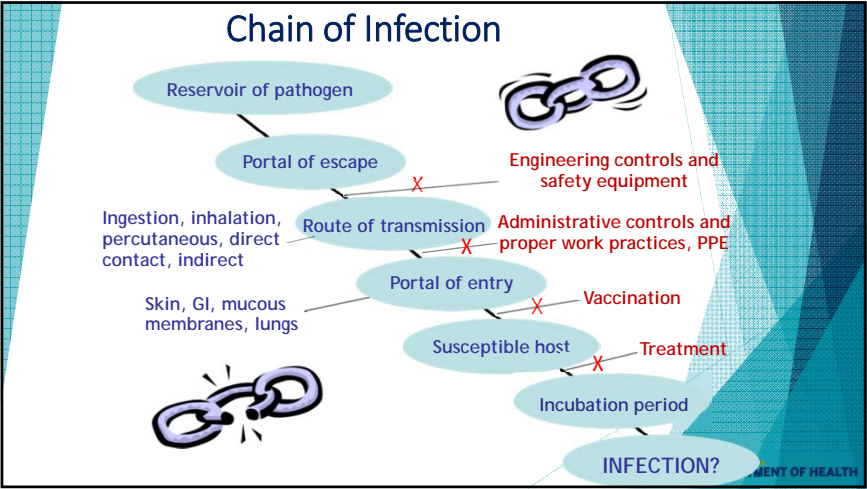
Principles of Biosafety

Risk assessment and containment can **reduce** occurrence of healthcare worker acquired infections by:

Reducing or minimizing exposure to microorganisms by breaking the “chain of infection”:

- ✓block routes of transmission
- ✓protect the portals of entry

But remember... the risk is never zero!



Portals of Entry

- Respiratory tract (lungs).....
- Gastrointestinal tract (mouth)....
- Mucous membranes (eyes, nose, mouth).....
- Non-intact skin.....
- Genitourinary tract.....
- Various.....

Routes of Transmission

- Inhalation of aerosols
- Ingestion
- Direct contact (splash, spill)
- Percutaneous (sharps, bites, vectors)
- Sexual
- Indirect (fomites*)

The images illustrate different transmission routes: a person coughing (respiratory), a hand holding a needle (percutaneous), and a mosquito (vector-borne).

**Fomite - an inanimate object (as a computer, doorknob, phone or work surface) that may be contaminated with infectious organisms and serve in their transmission.*

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Standard/Universal Precautions

*Standard (SP)/Universal Precautions (UP) are designed to reduce the risk of transmission of microorganisms **from both recognized and unrecognized sources** of infection.*

➢ SP are **more encompassing** and are not just for bloodborne pathogens - **handle all patient/specimens as if they are infectious**

- Hand hygiene
- PPE
- Safe sharps practices
- Safe handling of contaminated equipment or surfaces
- Respiratory hygiene

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Personal Protective Equipment (PPE)

Why?

- Protect the Healthcare Provider (HCP)
- Protect the patient

What?

- Gloves
- Respiratory protection
- Eye/face protection

***PPE does not eliminate the hazard- Know the limitations!**

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Respirators

- N95
- PAPR



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Respirators

IF respirators are indicated by the risk assessment:

- Personnel must have medical clearance, be fit tested and trained annually (OSHA 29 CFR 1910.134)
- They must be maintained
- They REDUCE exposure, do NOT eliminate exposure-risk is never zero
- Remember:
 - Facial hair can interfere with N95 seal
 - **Surgical masks are NOT respirators!**

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Aerosols and Droplets

➤ **Aerosol** -

- **Small particle** ($\sim < 0.5 \mu\text{m}$) that can remain suspended in the air and can be **inhaled deeply into the lung**.

➤ **Droplet** -


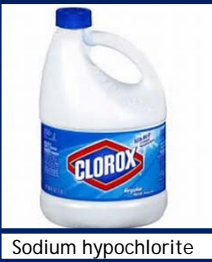

- **Larger particle** ($\sim > 0.5 \mu\text{m}$) that can **settle due to gravity**.
- May contaminate surfaces and be picked up on hands.



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Decontaminate Workspace

Maintain a clean workspace and decontaminate daily with a disinfectant that is effective against the target organism.



Sodium hypochlorite

70% Alcohol

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Effectiveness of Disinfectants

There is **no one universal disinfectant** effective against all organisms-because of:

- Concentration of the disinfectant
- Concentration of the agent
- Type of agent
- Contact time
- Amount of organic material
- Environmental conditions - pH, temp, humidity

Generalized Order of Resistance to Disinfection

- Prions-**Most Difficult**
- Bacterial spores (*B. anthracis*)
- Mycobacteria (*M. tuberculosis*)
- Nonlipid (non-enveloped) viruses (Norovirus, Poliovirus)
- Fungi (*Aspergillus*, *Candida albicans*)
- Vegetative bacteria (*S. aureus*)
- Lipid (enveloped) viruses (Ebola, HIV)-**Readily Killed**



Additional Recommendations

- Establish policies for **cell phone usage**
- Establish procedures for handling phones, keyboards, microscopes, etc.
- Remind clinicians to notify applicable personnel (e.g. nursing & lab staff) if a high risk organism is suspected



Exposure Response

In the event of an exposure, **immediate first aid** (i.e., an effective and timely cleansing response to a known wound) **may be the most critical determinant in preventing infection**



Exposure Response

- **Have plans and SOPs in place**
 - Available and accessible immediately and 24/7
 - Simple, easy to follow guidance
 - **Practice** emergency procedures with simulation drills
 - Review gaps and adjust
- **Are Medical Alert Cards in use?**
- **Are first aid kit contents checked regularly?**
- **Are exposures linked to further assessment and reporting (After Action Review Committees)?**



Staff Training Should Cover:

- Biohazards and hazard controls
- Risks of different types of exposures
- Available vaccinations and side effects
- Post-incident first aid and remediation
- Signs and symptoms of infection
- Emergency response procedures
- Incident reporting procedures



Staff Training

Promote benefits of non-punitive reporting of exposures and near misses:

- Use incident investigation in your training to accentuate the **“opportunity this presents”** not the **“failure it represents”**
- Case studies of real incidents
- CDC’s MMWR (Morbidity and Mortality Weekly Report)
www.cdc.gov/mmwr



Challenges in the Clinical Hospital

High stress

- Critical nature of work
- High workload and fast pace

Limited staff and resources (less \$\$) leads to more stress

- High workload, insufficient space, facility/infrastructure issues/breakdown

Lack of time or \$\$ for training (limited staff)

- Unsafe practices
- Assumption that isolation rooms and PPE are effective

Unfamiliar with agent (rare)

- Work conducted before risk is known

Lack of management support

- PPE usage not always enforced



Worker is pivotal in controlling the safe outcome of any operation!



Complacency & Routine can become the enemy!
Everyone has different perceptions and risk tolerance.



Antimicrobial Resistance Laboratory Network (ARLN)



- Established in 2016, CDC’s Antibiotic Resistance Laboratory Network (AR Lab Network) supports nationwide lab capacity to rapidly detect antibiotic resistance in healthcare, food, and the community, and inform local responses to prevent spread and protect people.
- Network includes seven regional labs, the National Tuberculosis Molecular Surveillance Center (National TB Center), and labs in 50 states, six cities, and Puerto Rico. As a whole, the network tracks changes in resistance and helps identify and respond to outbreaks faster.
- Network detects existing and emerging types of antibiotic resistance.



Antimicrobial Resistance Laboratory Network (ARLN)



- Tracks emerging resistance more effectively and generates stronger data to protect people and combat future resistance threats.
- This infrastructure will allow the public health community to rapidly detect emerging AR threats, sound the alarm for a comprehensive local response, and better understand these deadly threats so we can contain them quickly.
- The LDH Office of Public Health Microbiology Laboratory will participate and submit data to the ARLN.



Antimicrobial Resistance Laboratory Network (ARLN)

LDH OPH Laboratory Participation in the ARLN:

- LDH OPH Laboratory is currently validating test methods for confirmation and molecular characterization of Carbapenem resistant *Enterobacteriaceae* and *Pseudomonas aeruginosa*
- **Organisms to be tested -**
 - **Carbapenem Resistant Enterobacteriaceae (CRE)** - *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, and *Enterobacter* spp. that are resistant to imipenem, meropenem, doripenem, or ertapenem by standard susceptibility testing methods (i.e., minimum inhibitory concentrations of $\geq 4 \mu\text{g/mL}$ for doripenem, imipenem or meropenem or $\geq 2 \mu\text{g/mL}$ for ertapenem)
 - **Carbapenem Resistant Pseudomonas aeruginosa (CRPA)** - *P. aeruginosa* isolates that are resistant to imipenem, meropenem, or doripenem by standard susceptibility testing methods (i.e., minimum inhibitory concentrations of $\geq 8 \mu\text{g/mL}$)



Antimicrobial Resistance Laboratory Network (ARLN)

LDH OPH Laboratory Participation in the ARLN:

- LDH OPH Laboratory and ID Epidemiology are developing a pilot program with selected health care facilities throughout the state; submitting qualifying bacterial isolates.
- For isolates submitted, LDH OPH Laboratory will provide confirmatory testing for organism identification, susceptibility testing, phenotypic carbapenemase confirmation, and molecular characterization of carbapenemase producers.
- Confirmatory testing results will be provided to all participating laboratories.



Antimicrobial Resistance Laboratory Network (ARLN)

LDH OPH ARLN Test Menu:

➤ **Antimicrobial Susceptibility Testing (AST)** – confirmatory AST testing by disk diffusion for all isolates submitted. Antimicrobials tested include:

Drug class	CRE	CRPA
Carbapenems	2 carbapenems (ertapenem and either imipenem, doripenem or meropenem)	2 carbapenems (imipenem, doripenem or meropenem)
Cephems	ceftazidime and cefepime	ceftazidime and cefepime
B-lactam/B-lactamase inhibitor combinations	NA	piperacillin-tazobactam
Monobactams	aztreonam	aztreonam

- **Phenotypic Carbapenemase Confirmation by mCIM**
- **Molecular Characterization of Carbapenemase producers by PCR** (Cepheid GeneXpert Carba R Assay for detection of KPC, NDM, VIM, OXA-48, and IMP gene sequences)



Antimicrobial Resistance Laboratory Network (ARLN)

LDH OPH Laboratory/ARLN Result Reporting:

- State ARLN Labs will submit a monthly report of CRE and CRPA testing results to CDC. This data will allow for rapid detection of emerging AR threats and facilitate a rapid response to ensure containment.
- The LDH OPH Laboratory will report testing results back to submitting institutions within 2 working days via secure communications.
 - Please note:**
 - Results can be used to support infection prevention measures.
 - Results should not be a substitute for diagnostic procedures or used to guide clinical decisions.
- State Labs will report any novel and/or unusual AMR in CRE or CRPA to CDC.



Antimicrobial Resistance Laboratory Network (ARLN)

ARLN Goals:

- Detect new resistance and provide better big-picture trend tracking to create pathogen-specific solutions and support national public health strategies.
- Inform outbreak response when AR threats, like CRE, are reported, working together with state and local labs.
- Support innovations in antibiotic and diagnostic development. Samples from the labs will be made available through the CDC and FDA AR Isolate Bank, which researchers can use to develop earlier diagnoses and more effective treatment options.



Thank you for your time and attention!

Please contact us with questions:

- Jean-Jacques Aucoin, BS MT(ASCP)
Microbiology Laboratory Manager
jean-jacques.aucoin@la.gov
- LeAnn Covington, M. Ed., BS MT(ASCP)
Biosafety Program Advisor
leann.covington@la.gov



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